H4IIE bioassay-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQ) in fish collected in 2000 from the Texas Gulf coast of the United States.

Biomonitoring Environmental Status and Trends (BEST) and Environmental Monitoring and Assessment Program (EMAP) joint monitoring effort.

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TABLE OF CONTENTS

INTRODUCTION

The Environmental Protection Agency's Environmental Monitoring and Assessment Program EMAP Coastal 2000 program conducted a focused survey along the Texas coast during the summer of FY 2000 and FY 2001. The goal was to more fully characterize contaminant stressors in habitats associated with national parks and refuges by conducting several addition tests including the H4IIE bioassay of fish tissue. The H4IIE bioassay is one of the screening measures employed by the Biomonitoring of Environmental Status and Trends (BEST) Program to assess and characterize exposure to planar halogenated hydrocarbons (PHHs) as well as polycyclic aromatic hydrocarbons (PAHs). The H4IIE bioassay is a semi-quantitative assay that measures the overall toxic potency of PHHs and PAHs in the extracts of fish. PHHs consist largely of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). The information provided by the H4IIE bioassay complements the ecological metrics used in the EMAP Program. Texas Parks and Wildlife collected samples from approximately fifty sites on or within five kilometers of a national park or refuge along the Gulf Coast of Texas during the two year sampling period.

OBJECTIVE

1) To determine H4IIE EROD bioassay-derived 2,3,7,8-tetrachloro-*p*-dioxin equivalents (TCDD-EQ) in extracts of fish collected at selected sites along the Texas Gulf coast.

MATERIALS AND METHODS

Sample History

Benthic dwelling fish communities in coastal habitats associated with national parks or refuges along the Texas Gulf Coast were sampled in 2000 and 2001. Sampling locations and sample identification are summarized in Table 1. Samples were stored frozen at -20^{o} C for about 9 months until homogenized. Ground fish samples were stored at -40^{o} C for 5 days before shipping to the Columbia Environmental Research Center (CERC), Columbia, MO. Ground fish composites were packed into pesticide free, amber I-Chem® jars and shipped to CERC via Federal Express Overnight Shipping. Samples arrived in Coleman Coolers packed in Blue Ice and wrapped in plastic bubble wrap. Sample transmittal and chain of custody forms accompanied samples during transmission. Forty-eight composite fish samples arrived on 5/31/2001. Upon receipt at CERC, samples were unpacked, logged in by Jesse Arms (CERC) and stored at -15^{o} C until processed for extraction.

Analytical Sample Preparation Methods Summary:

All samples were assigned individual database identification numbers. Quality control (QC) samples (matrix blanks, procedural blanks, and positive control materials) were

prepared concurrently with the test samples. Positive control material was derived from samples of CERC's standard positive control matrix, common carp (*Cyprinus carpio*) tissue, collected from Saginaw Bay, Michigan, December 1988. Matrix blank material was derived from clean bluegill (*Lepomis macrochirus*) raised in CERC's holding pond. Samples were defrosted at room temperature and 10 or 20 g aliquots removed and combined with a, four-fold excess by weight, of anhydrous sodium sulfate. After dehydrating overnight, the mixture was homogenized in a blender, packed into an extraction column, and extracted with methylene chloride (CH_2Cl_2). The resultant extract was concentrated via rotary evaporation. The concentrated extract was then subjected to reactive clean up per CERC SOP P.186 (Appendix 1). The samples were then prepared for the H4IIE bioassay by concentrating to near dryness followed by transfer to 300 µL dosing vials through a series of three rinses, reconstitution and subsequent concentration cycles. The samples were reconstituted in a final volume of 150 µL in iso-octane and stored in capped conical dosing vials until used for the bioassay.

H4IIE Bioassay Method:

The H4IIE bioassay procedure was a modification of that reported by Tillitt et al. (1991). These modifications miniaturize and expedite the assay procedure, by allowing for sample processing in 96 well microtiter plates, reported in CERC SOP C5.194 (Appendix 1). The H4IIE rat hepatoma cell line (American Type Culture Collection, ATCC) was maintained using standard sterile tissue culture techniques. Cells were cultured in Dulbelcco's Modified Eagle's Medium (D-MEM) at 37^{o} C, 5% CO_2 (ambient chamber concentration). Cell cultures and exposures occurred in a humidified, temperature and carbon dioxide level-controlled incubator (Forma Scientific, Marietta, Ohio). Microtiter plates were seeded by pipetting 300 µL of a media/cell suspension (approximately 23,000 cells/mL) into each well. Post seeding, cells were allowed to proliferate for approximately 24 hours. The cell containing plates were then dosed with a serial dilution of TCDD or the samples and returned to the incubator for 72 hours to allow for maximal EROD induction. Upon completion of this induction period, the EROD reaction was measured for each well on each plate.

A standardized 2,3,7,8-tetrachlorodibenzo-*p*-dioxin solution was used to generate an analytical dose-response curve to which all other samples would be related. Generally, six standard dose-response curves were measured on each assay date. Dose-response curves were prepared as a set of 7 serial dilutions along with an iso-octane blank for each sample or standard. The TCDD standard, (10 pg TCDD/µL iso-octane), was diluted in a ratio of 1:2 (v/v) while each experimental sample was diluted 1:3 (v/v). Six basal curves, non-dosed cells, to which were assigned an artificial dose were, also, run on each assay date. These were used to calculate basal induction, limits of detection (LOD), and limits of quantitation (LOQ) for each assay date. The TCDD standard and basal curves were placed in varying plate positions and interspersed among sample curves. All experimental fluorometric data were collected with a Perkin-Elmer BioSystems Cytofluor 4000 instrument.

A resorufin standard curve (range, 0 to 320 pmol) was generated on each assay date using the following procedure. Eight standard solutions were prepared by making a 1:1 (v/v) serial dilution of a prepared 16 μM resorufin/phosphate buffered saline (PBS) working stock. This working stock was prepared by making a dilution of a 200 μM, resorufin/methanol, super stock. The concentration of the super stock was checked spectrophotometrically at 571 nm on each assay date. Six replicates of each resorufin standard were added to a plate and the average background corrected arbitrary fluorescence units (AFU's) were plotted against the nominal resorufin concentrations to produce the resorufin standard curve and linear regression equation.

The reaction was initiated in experimental plates and fluorescence resulting from resorufin formation in each well was monitored once a minute for 20 minutes. The background corrected AFU's for the experimental plates were compared to the corresponding linear fit of the eight point resorufin standard curve and AFU's were converted into pmol of resorufin formed. The resorufin content in each well was plotted against time to evaluate any deviations from linearity in the progressive formation of resorufin with time. A linear regression analysis was performed on each sample well to obtain the slope and estimate the rate of reaction (pmol/min). The reaction rate observed in each well was normalized according to the measured protein content, generating a value of specific activity in units of pmols resorufin formed/(min*mg) of protein. Reported results are the average of at least four replicate curves. The linear portions of the slopes derived from each of these curves were normalized to the average initial slope obtained for the TCDD standard curves, resulting in a measure of an equivalent dose of TCDD (TCDD-EQ) for each sample.

The protein content in each well was determined using a fluorescamine-based protein assay (Undenfriend et al. 1972; Bohlen et al. 1973; Lorenzen and Kennedy, 1993). The reaction was allowed to progress for 10 minutes then fluorescence measurements were made. A separate BSA standard curve (range, 0 to 120 μg) was generated for each assay day. Eight standard solutions were prepared by making a 1:1 (v/v) serial dilution of a prepared 6 mg/mL BSA stock. Six replicates of each BSA standard were added to a plate and the average background corrected arbitrary fluorescence units (AFU's) were plotted against the nominal BSA concentrations to produce the standard curve and linear regression equation. Fluorescence values were measured for each sample well, the background corrected AFU's were compared to the corresponding linear fit of the eight point BSA standard curve and AFU's were converted into mg of protein.

All EROD assay reagents were incubated for ten minutes at 37^{o} C prior to data collection. The correct sample identification and its associated microtiter plate well were recorded on data log sheets and stored with the laboratory notebook. All electronic files were stored on CD's with names and other pertinent information recorded in the laboratory notebook.

Excess of the tissue extracts was stored at room temperature in sealed conical vials with the volume marked.

Quality Assurance and Quality Control:

The objective of the quality assurance plan of this study was to ensure that the biochemical analyses were accurate and representative measures of the TCDD-EQs found in each composite sample generated from those collected in the field portion of this study. The general scheme included replication of assayed samples, comparison of calibration against known standards, proper maintenance and calibration of equipment, accurate sample tracking and chain of custody, proper documentation at all steps of sample processing and other considerations of Good Laboratory Practice (GLP). The specific aspects of the QA plan related to the H4IIE EROD assay are given below.

All experimental information was recorded in bound notebooks and copies maintained in a separate, secured area. Instrument printouts and computer-generated data tables were uniquely labeled and cross-referenced to the project notebook. The accuracy of all such data reductions was independently verified. Hard copies of computerized data files were maintained in a project notebook. Computer files were backed-up and archived on CD's. All equipment used in this study was routinely inspected and preventive maintenance performed. A logbook was kept for each instrument to document its use, performance and maintenance.

Replication and subsequent performance checks were performed at many stages of the H4IIE EROD assay procedure. A composite TCDD dose-response curve was generated from the average of 6 independent determinations for each composite sample. Ten percent (10%) of tissue extract samples were assayed in triplicate, as were all positive control and some matrix blank samples. Eight-point resorufin and BSA (bovine serum albumin) standard curves were prepared at 6 replicates for each concentration, and analyzed concurrently with the TCDD standards and samples. Positive control fish tissue extracts were analyzed on each assay date along with the samples. The source and lot number of the BSA and resorufin were recorded in the laboratory notebook. Scatter plots for the resorufin (Figure 1) and BSA (Figure 2) standard curves have been included with this report. These scatter plots were prepared to demonstrate the consistency of the fluorescence response with concentration over the time course of sample evaluation. They were also used to facilitate data analysis. The slope and y-intercept values were recorded electronically.

The concentrations of the resorufin, ethoxyresorufin and NADPH reagents were checked on each assay date using a spectrophotometer and their actual concentrations determined based upon Beer's Law using known extinction coefficients for the different reagents. It was deemed acceptable if the actual concentration was within 10% of the nominal concentration.

Positive control, matrix blank, and procedure blank tissue extracts were included along with the samples for H4IIE analysis on each assay date to assure that both the EROD enzyme assay and the reagents were behaving according to specifications. The positive control was prepared from CERC's reference material, common carp from Saginaw Bay, Michigan. Five 10g aliquots were separately extracted and carried through reactive

absorbent clean-up columns in accordance with CERC's SOP P.186 (Appendix 1). Positive control extracts were designated with their own unique tracking number. Matrix blank extracts from pond-raised bluegill and procedure blank extracts were prepared in the same process as that used for the positive control and were assigned a unique tracking number that was matched with a positive control sample.

Data Analysis:

The standard curve data for both the resorufin and BSA standard curves were separately compiled and each plotted as a set, in order to verify the consistency and consequently the reliability of the sample data as a whole. In each case, a linear regression, 95% confidence interval, and prediction interval were plotted (Figures 1 and 2, respectively). The means and error limits (SD or standard deviation, and CV or coefficient of variation) for both the resorufin and BSA standard data are given below.

	slope		y intercept	
	mean	(SD, CV)	mean	(SD, CV)
Resorufin	88.8 AFUs/pmol	(8, 9%)	-72.3 AFUs	(226, 312%)
BSA	380 AFUs/mg	(43, 11%)	1532 AFUs	(411, 27%)

The time courses for the production of resorufin (i.e. the EROD reaction rates) were evaluated graphically to ensure linearity of the response. Linearity of the reactions verifies that non-saturating substrate levels or enigmatic kinetics did not limit the reactions. In cases where the kinetic reaction did not yield a linear response over the 20-minute sampling time, the linear portion of the curve was used to calculate the rate of reaction.

The linear portion of a plot of EROD specific activity versus gram tissue equivalents per mg cellular protein was used to determine the EROD induction response for the H4IIE cells for a given sample. This measure of EROD induction was translated into an equivalent dose of 2,3,7,8-tetrachloro-*p*-dioxin, TCDD, by dividing the average induction response arising from treatment of the cells with sample by that response arising from treatment of cells with the standard, TCDD (Eq. 1). The resultant metric for potency estimates was TCDD-equivalents (TCDD-EQs) in the extract or tissue sample.

$$\text{TCDD-EQ (pg/g)} = [(\text{EROD/g-equivalent in extract})/(\text{EROD/pg TCDD})] \quad (1)$$

Positive control, matrix blank and procedure blank samples were included during each data collection and work-up phase of this study. Three dose-response curves were run and evaluated for the positive control sample on each assay date. A composite dose-response curve was developed for the positive control samples used on each assay date. These were generated by taking the average of individual dose-response curves. The mean slope and standard deviation were calculated. The positive control samples assayed in this study were plotted as TCDD-EQ versus assay date. These are illustrated in combination with line plots indicating the lab mean positive control value, high control

limit (mean + 2 X SD), and low control limit (mean – 2 X SD) (Figure 3). Data for matrix blank and procedure blank samples were prepared in the same manner and illustrated in separate plots (Figure 4, and 5, respectively).

The degree of EROD induction in reagent blanks and basal cells were determined in addition to the measurement of EROD induction for the TCDD standard and positive controls. The limits of detection (LOD) and quantitation (LOQ) for each assay date were calculated, as described by Keith et al. (1983). These parameters were calculated from the observed level of basal EROD activity measured in the H4IIE cells on a given day. The LOD was defined to be equal to the average basal activity plus 3 times the standard deviation of the mean (standard error) associated with that activity. The LOQ was defined to be equal to the average basal activity plus 10 times the standard deviation of the mean (standard error) associated with that activity. These measures were used to evaluate the sample data results and to determine whether they were detectable or measurable above that of the background. Based upon the basal level of EROD activity found in uninduced H4IIE cells, the criteria of LOD and LOQ could be used to judge the significance of the measured results obtained for the samples. Control charts that evaluate the run-to-run variation of LOD and LOQ are presented (Figure 6). In each case, the value for each assay date is plotted in combination with line plots indicating the lab mean value, high confidence limit (mean + 2 X SD), and low confidence limit (mean – 2 X SD).

Replicates of sample data were treated as separate samples until TCDD-EQ's were calculated. These were then averaged for reporting in tables and are indicated. A sample was re-run if the coefficient of variation was over 25%, starting with the concentrated tissue extract in iso-octane. Exceptions were made in the cases of the blanks and basal measurements or when the measured values fell below or very close to the limit of quantitation.

The H4IIE data of individual samples or mean data for those sites from which were assayed two or more samples were presented graphically on a map (Figure 7).

RESULTS and DISCUSSION

Quality Assurance

The data generated for all resorufin standard curves were compiled and plotted as background-corrected fluorescence values versus resorufin content per well (Figure 1). The linear regression, 95% confidence interval, and prediction interval for all of the data were plotted in Sigma Plot. As seen in the previous table the y-intercept coefficient of variation (CV), was 312% while that for the slope was 9%. While the y-intercept accounts for the day-to-day background fluctuations in the instrument, the slope reveals the correlation between fluorescence and resorufin content. Since the associated error in the slope was low, a high degree of confidence may be placed in the reliability of the mathematical conversion between measured fluorescence and calculated resorufin content throughout the course of the study.

The data generated for all BSA standard protein curves were compiled and plotted in the same manner as the resorufin with background-corrected fluorescence values versus protein content per well (Figure 2). The y-intercept coefficient of variation (CV) was 27% while that for the slope was 11%.

Generally, the results for all samples fell within the 25% CV limit as set forth by the QA objectives. Those samples that exceeded the 25% CV maximum fell into the reagent blank or basal classes of sample measurements or were samples that had calculated TCDD-EQs on the order of LOQ or less.

In order to evaluate the reproducibility of the experimental method throughout the time course of the analysis, the TCDD-EQs determined for the positive control, matrix blank and procedure blank samples were plotted as stated above (Figure 3, 4, and 5). All positive control, matrix blank, and procedure blank samples fell within set confidence intervals of the lab average.

Examination of the LOD and LOQ (Figure 6) control chart illustrates that all values fell within limits.

Hazards of TCDD-EQ in the Aquatic Ecosystem

The H4IIE bioassay responds to chemicals that bind to the aryl hydrocarbon receptor (Ah-R). The chemicals included in this class are polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polycyclic aromatic hydrocarbons (PAHs). The H4IIE bioassay integrates the overall potency of these chemicals. The response of the cells to the extract of the environmental sample (fish in this case) is calibrated against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the resultant potency is given as TCDD-equivalents (TCDD-EQs). The extraction procedures used for preparation of the samples in this monitoring effort were designed to remove PAHs during the extraction and clean-up steps. Subsequently, the potency estimates of dioxin-like activity in fish derived from these tests are representative of the amounts of PCBs, PCDDs, and PCDFs in the samples. Other compounds may have been present in these fish and could have elicited a response in the H4IIE cells, however; PCBs, PCDDs, and PCDFs (collectively referred to as planar halogenated hydrocarbons or PHHs) are commonly the predominant chemical contaminants found in environmental samples (Giesy et al. 1994). The results of the H4IIE bioassay are to be used as a screening tool for the overall potency of dioxin-like chemicals present in the fish samples. The results can be used to prioritize further analysis or monitoring efforts, and make simple predictions about the relative risks expected from this class of compounds.

Results of the H4IIE bioassay can be categorized based on concerns due to dioxin-like effects. The H4IIE bioassay is a screen for dioxin-like potency observed in extracts of biotic and even abiotic environmental samples. The results of the assay are an integration of the total of the dioxin-like chemicals present in the extracts. The amounts of individual chemicals present in the environmental samples are not delineated with this

assay. Chemical-specific risk assessments require traditional analytical chemistry data on individual chemicals. However, the data from the H4IIE bioassay can be used to conduct hazard ranking of the sites in this monitoring program. The hazards of dioxin-like chemicals are generally observed in species at the top of the trophic chain (ie. fish-eating birds and mammals). There are numerous toxic endpoints associated with dioxin-like chemicals, but the endpoints which are the most sensitive and the endpoints which are the most ecologically relevant to assessment of fish and wildlife populations are the reproductive effects (Peterson et al. 1993). In particular, rates of embryo lethality and birth deformities are important for understanding the effects of these compounds on a population. The results of the H4IIE bioassay were categorized to reflect the relative hazard that might be expected from the dioxin potency measured in the fish samples. The categories are relative benchmarks for comparative purposes and should not be construed as definitive thresholds of toxicity.

The relative hazard categories for the H4IIE bioassay results were based upon toxicity of the most sensitive life stage to the effects of dioxins, the developing embryo or fetus and the expected potential for biomagnification. Dioxins and other PHHs are known to biomagnify from fish to fish-eating birds and fish-eating mammals. Toxicity reference values (TRVs) of TCDD in fish and wildlife range from 35 pg TCDD/g egg in fish based on lake trout early life stage (ELS) mortality (Walker et al. 1994), to 100 pg TCDD/g egg for avian embryo lethality taken from a feeding study with ring-necked pheasant (Nosek et al. 1993). Reproductive toxicity of TCDD in mink occurs at 60 pg TCDD/g of liver in the adult mink (Tillitt et al. 1996). Taken together with the degree of biomagnification expected, a hazard category may be developed. The results of the H4IIE bioassay represent all of the PHHs present in the sample. These chemicals have a range of bioaccumulation or biomagnification potentials and for this reason, a chemical-specific risk assessment is not possible. However, if the potency of TCDD-EQs as measured by the H4IIE bioassay were evaluated as if they were derived from TCDD alone, then relative hazard categories may be developed. It is known that other Ah-R agonists, such as PCBs, are ubiquitous in the environment. The biomagnification factors for these other chemicals that contribute to the whole mixture of dioxin-like chemicals are sometimes greater and sometimes less than that of TCDD. Uses of the TCDD values simply provide a reference point from which to make relative screening categories. The biomagnification factor of TCDD from forage fish into predatory fish is approximately 1.0 (Cook et al. 1993; Jones et al. 2001). The biomagnification factor of TCDD from fish into the eggs of a fish-eating bird is approximately 20 (Braune and Norstom, 1989) and the biomagnification factor of TCDD from fish into mink livers is approximately 11 (Tillitt et al. 1996). Hazard categories may be developed from simple division of the toxicity reference values by the biomagnification factors (ie. TRV/BMF). Fish health may be expected to be impaired when TCDD-EQs in fish are 35 pg/g (TRV/BMF = 35/1). Avian reproductive health may be expected to be impaired when TCDD-EQs in fish are 5 pg/g (TRV/BMF = 100/20), while wildlife reproductive health can be expected to be impaired when TCDD-EQs in fish are 5 pg/g (TRV/BMF = 60/11). The TRV estimated by Tillitt et at. (1996) for dietary concentrations of TCDD equivalents in fish to protect mink reproductive health was 4.4 pg TCDD-EQ/g of fish. Thus, based on the potential for reproductive impairment and these biomagnification factors, H4IIE bioassay

values in fish greater than 5 pg TCDD-EQ/g may be hazardous to avian and mammalian wildlife which consume fish. Therefore, general hazard categories for the concentrations of TCDD-EQs in fish have been set as: 1) not expected to be hazardous (LOD to LOQ); 2) potentially hazardous (measured values < 5 pg TCDD-EQs/g); and 3) likely to be hazardous (≥ 5 pg TCDD-EQs/g). The H4IIE bioassay data from these analyses were presented graphically based on these categories (Fig. 7).

TCDD-EQs in Fish

All extracts of fish collected from sites along the Texas Gulf Coast had dioxin-like potencies that were less than 5 pg TCDD-EQs/g. These values indicate no hazard or a low potential hazard to fish eating birds & wildlife (Tables 2 & 3, Figure 7). The three extracts from the Sabine Lake System were all below LOQ. The twelve Galveston Bay System sites yielded extracts that were mostly below LOQ. Three of the sites had dioxin-like potencies in the potentially hazardous range. These sites were Buffalo Bayou (TX00-0001), with a TCDD-EQ of 2.3 pg/g, Basford Bayou (TX00-0002), with a TCDD-EQ of 0.8 pg/g, and Upper Galveston Bay (TX00-0011), with a TCDD-EQ of 2.4 pg/g. Extracts from eight sites in the Matagorda Bay System were analyzed. All but two were below LOQ and those in the measurable range were both below 1 pg/g, which is at the low end of the potentially hazardous category. The three San Antonio Bay site extracts had very low dioxin-like potencies, two were below LOQ and site TX00-0038 had a TCDD-EQ of 0.5 pg/g. All four Aransas Bay System sites resulted in low TCDD-EQs. Only site Mesquite Bay (TX00-0039), from which two samples were taken, analyzed separately and then averaged, had a measurable TCDD-EQ value of 0.1 pg/g. The same scenario occurred for the four sites in the Corpus Christi Bay System. The two sample site was TX00-0041 and it, also, had a very low average measured TCDD-EQ of 0.1 pg/g. The Upper Laguna Madre System only had one site, Baffin Bay (TX00-0043), with a measurable TCDD-EQ of 0.5 pg/g and all sites from the Lower Laguna Bay System were below LOD. A second sample from the Upper Laguna Madre System, site Baffin Bay (TX00-0046), was listed in the collection, however, there was no sample in the jar received at CERC, and so no analysis was completed for that sample.

Summary and Conclusions

Forty-seven sites along the Texas Gulf coast on or within five kilometers of a national park or refuge were sampled to collect benthic dwelling organisms so dioxin-like contamination in these samples could be determined. The relative potency of dioxin-like chemicals found in these samples was assessed with a bioassay screening system, the H4IIE rat hepatoma cell line. The H4IIE bioassay measures all of the chemicals in an organic extract that bind to the dioxin receptor and cause dioxin-like toxicity. The results of the H4IIE bioassay may be used to highlight areas or environments in which dioxins or dioxin-like chemicals may be of concern. The H4IIE bioassay can be used to indicate those environments which need further chemical analysis, from those environments that do not need further chemical characterization for this class of compounds. The H4IIE bioassay results were categorized into 2,3,7,8-

tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-EQs) that were thought to be 1)no to low hazard; 2) potentially hazardous; 3) likely to be hazardous.

The dioxin-like potencies of the extracts of samples collected on or near national parks or refuges along the Texas Gulf coast were quite low. Two of the samples from the Galveston Bay area resulted in TCDD-EQs that were in the middle of the potentially hazardous range.

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Table 1. Geographic location of EMAP sampling sites along the Texas Gulf Coast.

Station	Sample ID	Sampling Site	Latitude (°N)	Longitude (°W)	Species
TX00-0001	23080	Galveston Bay System, Buffalo Bayou	29º 45' 18.00"	95 18' 36.00"	White Shrimp
TX00-0002	23081	Galveston Bay System, Basford Bayou	29º 17' 45.60"	94º 57' 32.40"	Spot, Perch
TX00-0009	23082	Lower Laguna Madre System, Lower Laguna Madre	26º 14' 56.40"	97º 14' 09.60"	Toad Fish
TX00-0010	23083	Galveston Bay System, Trinity Bay	29º 40' 30.00"	94º 46' 30.00"	Catfish
TX00-0011	23084	Galveston Bay System, Upper Galveston Bay	29º 33' 28.80"	94º 59' 31.20"	Spot, Shrimp
TX00-0012	23085	Galveston Bay System, Trinity Bay	29º 43' 30.00"	94º 48' 28.80"	Atlantic Croaker/Catfish
TX00-0013	23086	Galveston Bay System, Trinity Bay	29º 37' 30.00"	94º 49' 30.00"	Atlantic Croaker
TX00-0014	23087	Matagorda Bay System, Lavac Bay	29º 34' 30.00"	96º 32' 31.20"	Penaeid Shrimp
TX00-0015	23088	Matagorda Bay System, Carancahua Bay	28º 36' 31.20"	96º 23' 31.20"	Atlantic Croaker
TX00-0015	23089	Matagorda Bay System, Carancahua Bay	28º 36' 31.20"	96º 23' 31.20"	Catfish
TX00-0016	23090	Matagorda Bay System, Matagorda Bay	28º 34' 30.00	96º 21' 28.80"	Atlantic Croaker
TX00-0017	23091	Matagorda Bay System, Matagorda Bay	28º 31' 30.00"	96º 29' 31.20"	Pinfish
TX00-0018	23092	San Antonio Bay System, San Antonio Bay	28º 18' 28.80"	96º 47' 31.20"	Catfish
TX00-0019	23093	Aransas Bay System, Aransas Bay	28º 03' 28.80"	97º 01' 30.00"	Atlantic Croaker
TX00-0020	23094	Aransas Bay System, St. Charles Bay	28º 11' 31.20"	96º 56' 31.20"	Atlantic Croaker
TX00-0021	23095	Aransas Bay System, Copano Bay	28º 08' 31.20"	97º 05' 31.20"	Atlantic Croaker
TX00-0022	23096	Corpus Christi Bay System, Corpus Christi Bay	27º 46' 30.00"	97º 20' 31.20"	Atlantic Croaker
TX00-0023	23097	Corpus Christi Bay System, Corpus Christi Bay	27º 49' 30.00"	97º 21' 28.80"	Atlantic Croaker
TX00-0024	23098	Matagorda Bay System, East Matagorda Bay	28º 41' 20.40"	95º 54' 28.80"	Atlantic Croaker
TX00-0025	23099	Sabine Lake System, Sabine Lake	29º 50' 31.20"	93º 48' 28.80"	Atlantic Croaker
TX00-0026	23100	Sabine Lake System, Sabine Lake	29º 49' 30.00"	93º 53' 31.20"	Atlantic Croaker
TX00-0027	23101	Sabine Lake System, Keith Lake	29º 45' 28.80"	93º 57' 28.80"	Hardhead/Atlantic Croaker
TX00-0028	23102	Galveston Bay System, East Bay	29º 29' 31.20"	94º 43' 30.00"	Atlantic Croaker
TX00-0029	23103	Galveston Bay System, Lower Galveston Bay	29º 25' 30.00"	94º 52' 30.00"	Atlantic Croaker
TX00-0030	23104	Galveston Bay System, West Bay	29º 21' 28.80"	94º 52' 30.00"	Pinfish/Atlantic Croaker
TX00-0031	23105	Galveston Bay System, East Bay	29º 31' 30.00"	94º 40' 30.00"	Atlantic Croaker
TX00-0032	23106	Galveston Bay System, East Bay	29º 29' 31.20"	94º 38' 31.20"	Atlantic Croaker
TX00-0033	23107	Galveston Bay System, Lower Galveston Bay	29º 23' 31.20"	94º 47' 31.20"	Pinfish

Table 1. Geographic location of EMAP sampling sites along the Texas Gulf Coast.

Station	Sample ID	Sampling Site	Latitude (°N)	Longitude (°W)	Species
TX00-0034	23108	Matagorda Bay System, Matagorda Bay	28º 35' 31.20"	96º 07' 30.00"	Catfish
TX00-0035	23109	Matagorda Bay System, Matagorda Bay	28º 31' 30.00"	96º 21' 28.80"	Atlantic Croaker
TX00-0036	23110	Matagorda Bay System, Matagorda Bay	28º 31' 30.00"	96º 17' 31.20"	Atlantic Croaker
TX00-0037	23111	San Antonio Bay System, Shoalwater Bay	28º 21' 28.80"	96º 34' 30.00"	Catfish
TX00-0038	23112	San Antonio Bay System, San Antonio Bay	28º 12' 28.80"	96º 44' 31.20"	Penaeid Shrimp
TX00-0039	23113	Aransas Bay System, Mesquite Bay	28º 09' 28.80"	96º 52' 30.00"	Gafftop Sail Catfish
TX00-0039	23114	Aransas Bay System, Mesquite Bay	28º 09' 28.80"	96º 52' 30.00"	Atlantic Croaker
TX00-0040	23115	Corpus Christi Bay System, Corpus Christi Bay	27º 47' 31.20"	97º 11' 31.20"	Silver Perch
TX00-0041	23116	Corpus Christi Bay System, Corpus Christi Bay	27º 47' 31.20"	97º 07' 30.00"	Atlantic Croaker
TX00-0041	23117	Corpus Christi Bay System, Corpus Christi Bay	27º 47' 31.20"	97º 07' 30.00"	Pinfish
TX00-0042	23118	Upper Laguna Madre System, Upper Laguna Madre	27º 27' 28.80"	97º 19' 30.00"	Silver Perch
TX00-0043	23119	Upper Laguna Madre System, Baffin Bay	27º 16' 30.00"	97º 32' 31.20"	Atlantic Croaker
TX00-0044	23120	Upper Laguna Madre System, Upper Laguna Madre	27º 40' 30.00"	97º 14' 31.20"	Pinfish
TX00-0045	23121	Upper Laguna Madre System, Upper Laguna Madre	27º 32' 31.20"	97º 19' 30.00"	Pinfish
TX00-0046	23123	Upper Laguna Madre System, Baffin Bay	27º 16' 30.00"	97º 37' 30.00"	Catfish
TX00-0047	23124	Lower Laguna Madre System, Lower Laguna Madre	26º 36' 28.80"	97º 24' 28.80"	Catfish
TX00-0048	23125	Lower Laguna Madre System, Lower Laguna Madre	26º 32' 31.20"	97º 23' 31.20"	Pigfish
TX00-0049	23126	Lower Laguna Madre System, Lower Laguna Madre	26º07' 30.00"	97º 14' 31.20"	Toadfish
TX00-0050	23127	Lower Laguna Madre System, Lower Laguna Madre	26º46' 30.00"	97º 27' 28.80"	Pinfish

Table 2. TCDD equivalents (pg/g) of EMAP samples taken along the Texas Gulf Coast. Extraction/assay triplicates were samples that were extracted in triplicate and then one of those were dosed in the assay in triplicate. Assay triplicate are those samples that were dosed in the assay in triplicate.

Station	Sample ID	Sampling Site	Species	TCDD-EQ (pg/g)	SD	Notes
TX00-0001	23080	Galveston Bay System, Buffalo Bayou	White Shrimp	2.3	0.5	
TX00-0002	23081	Galveston Bay System, Basford Bayou	Spot, Perch	0.8	0.3	
TX00-0009	23082	Lower Laguna Madre System, Lower Laguna Madre	Toad Fish	<LOD		Extraction/Assay triplicate
TX00-0010	23083	Galveston Bay System, Trinity Bay	Catfish	<LOQ		
TX00-0011	23084	Galveston Bay System, Upper Galveston Bay	Spot, Shrimp	2.4	0.5	
TX00-0012	23085	Galveston Bay System, Trinity Bay	Atlantic Croaker/Catfish	<LOD		
TX00-0013	23086	Galveston Bay System, Trinity Bay	Atlantic Croaker	<LOD		Assay triplicate
TX00-0014	23087	Matagorda Bay System, Lavac Bay	Penaeid Shrimp	<LOD		
TX00-0015	23088	Matagorda Bay System, Carancahua Bay	Atlantic Croaker	<LOQ		
TX00-0015	23089	Matagorda Bay System, Carancahua Bay	Catfish	1.0	0.3	
TX00-0016	23090	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	<LOD		
TX00-0017	23091	Matagorda Bay System, Matagorda Bay	Pinfish	<LOD		
TX00-0018	23092	San Antonio Bay System, San Antonio Bay	Catfish	<LOQ		Extraction/Assay triplicate
TX00-0019	23093	Aransas Bay System, Aransas Bay	Atlantic Croaker	<LOQ		
TX00-0020	23094	Aransas Bay System, St. Charles Bay	Atlantic Croaker	<LOQ		
TX00-0021	23095	Aransas Bay System, Copano Bay	Atlantic Croaker	<LOQ		
TX00-0022	23096	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker	<LOD		
TX00-0023	23097	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker	<LOQ		
TX00-0024	23098	Matagorda Bay System, East Matagorda Bay	Atlantic Croaker	<LOD		Assay triplicate
TX00-0025	23099	Sabine Lake System, Sabine Lake	Atlantic Croaker	<LOQ		
TX00-0026	23100	Sabine Lake System, Sabine Lake	Atlantic Croaker	<LOD		
TX00-0027	23101	Sabine Lake System, Keith Lake	Hardhead/Atlantic Croaker	<LOD		Extraction/Assay triplicate
TX00-0028	23102	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		
TX00-0029	23103	Galveston Bay System, Lower Galveston Bay	Atlantic Croaker	<LOD		
TX00-0030	23104	Galveston Bay System, West Bay	Pinfish/Atlantic Croaker	<LOD		
TX00-0031	23105	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		
TX00-0032	23106	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		

Table 2. TCDD equivalents (pg/g) of EMAP samples taken along the Texas Gulf Coast. Extraction/assay triplicates were samples that were extracted in triplicate and then one of those were dosed in the assay in triplicate. Assay triplicate are those samples that were dosed in the assay in triplicate.

Station	Sample ID	Sampling Site	Species	TCDD-EQ (pg/g)	SD	Notes
TX00-0033	23107	Galveston Bay System, Lower Galveston Bay	Pinfish	<LOD		
TX00-0034	23108	Matagorda Bay System, Matagorda Bay	Catfish	<LOD		
TX00-0035	23109	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	<LOD		
TX00-0036	23110	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	0.3	0.3	
TX00-0037	23111	San Antonio Bay System, Shoalwater Bay	Catfish	<LOD		
TX00-0038	23112	San Antonio Bay System, San Antonio Bay	Penaeid Shrimp	0.5	0.4	
TX00-0039	23113	Aransas Bay System, Mesquite Bay	Gafftop Sail Catfish	<LOD		
TX00-0039	23114	Aransas Bay System, Mesquite Bay	Atlantic Croaker	0.2	0.2	Assay triplicate
TX00-0040	23115	Corpus Christi Bay System, Corpus Christi Bay	Silver Perch	<LOD		
TX00-0041	23116	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker	<LOD		
TX00-0041	23117	Corpus Christi Bay System, Corpus Christi Bay	Pinfish	0.4	0.1	Extraction/Assay triplicate
TX00-0042	23118	Upper Laguna Madre System, Upper Laguna Madre	Silver Perch	<LOD		
TX00-0043	23119	Upper Laguna Madre System, Baffin Bay	Atlantic Croaker	0.5	0.3	
TX00-0044	23120	Upper Laguna Madre System, Upper Laguna Madre	Pinfish	<LOD		
TX00-0045	23121	Upper Laguna Madre System, Upper Laguna Madre	Pinfish	<LOD		
TX00-0046	23123	Upper Laguna Madre System, Baffin Bay	Catfish	<LOD		Assay triplicate
TX00-0047	23124	Lower Laguna Madre System, Lower Laguna Madre	Catfish	<LOD		
TX00-0048	23125	Lower Laguna Madre System, Lower Laguna Madre	Pigfish	<LOD		Extraction/Assay triplicate
TX00-0049	23126	Lower Laguna Madre System, Lower Laguna Madre	Toadfish	<LOD		
TX00-0050	23127	Lower Laguna Madre System, Lower Laguna Madre	Pinfish	<LOD		

Table 3. Mean TCDD equivalents (pg/g) of EMAP stations along the Texas Gulf Coast

Station	Sampling Site	Species	TCDD-EQ (pg/g)	SD	*n*	range
TX00-0001	Galveston Bay System, Buffalo Bayou	White Shrimp	2.3	0.5	1	
TX00-0002	Galveston Bay System, Basford Bayou	Spot, Perch	0.8	0.3	1	
TX00-0009	Lower Laguna Madre System, Lower Laguna Madre	Toad Fish	<LOD		1	
TX00-0010	Galveston Bay System, Trinity Bay	Catfish	<LOQ		1	
TX00-0011	Galveston Bay System, Upper Galveston Bay	Spot, Shrimp	2.4	0.5	1	
TX00-0012	Galveston Bay System, Trinity Bay	Atlantic Croaker/ Catfish	<LOD		1	
TX00-0013	Galveston Bay System, Trinity Bay	Atlantic Croaker	<LOD		1	
TX00-0014	Matagorda Bay System, Lavac Bay	Penaeid Shrimp	<LOD		1	
TX00-0015	Matagorda Bay System, Carancahua Bay	Atlantic Croaker/Catfish	0.7	0.3	2	<LOQ - 1.0
TX00-0016	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	<LOD		1	
TX00-0017	Matagorda Bay System, Matagorda Bay	Pinfish	<LOD		1	
TX00-0018	San Antonio Bay System, San Antonio Bay	Catfish	<LOQ		1	
TX00-0019	Aransas Bay System, Aransas Bay	Atlantic Croaker	<LOQ		1	
TX00-0020	Aransas Bay System, St. Charles Bay	Atlantic Croaker	<LOQ		1	
TX00-0021	Aransas Bay System, Copano Bay	Atlantic Croaker	<LOQ		1	
TX00-0022	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker	<LOD		1	
TX00-0023	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker	<LOQ		1	
TX00-0024	Matagorda Bay System, East Matagorda Bay	Atlantic Croaker	<LOD		1	
TX00-0025	Sabine Lake System, Sabine Lake	Atlantic Croaker	<LOQ		1	
TX00-0026	Sabine Lake System, Sabine Lake	Atlantic Croaker	<LOD		1	
TX00-0027	Sabine Lake System, Keith Lake	Atlantic Croaker	<LOD		1	
TX00-0028	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		1	
TX00-0029	Galveston Bay System, Lower Galveston Bay	Atlantic Croaker	<LOD		1	
TX00-0030	Galveston Bay System, West Bay	Pinfish/Atlantic Croaker	<LOD		1	
TX00-0031	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		1	
TX00-0032	Galveston Bay System, East Bay	Atlantic Croaker	<LOD		1	
TX00-0033	Galveston Bay System, Lower Galveston Bay	Pinfish	<LOD		1	

Table 3. Mean TCDD equivalents (pg/g) of EMAP stations along the Texas Gulf Coast

Station	Sampling Site	Species	TCDD-EQ (pg/g)	SD	*n*	range
TX00-0034	Matagorda Bay System, Matagorda Bay	Catfish	<LOD		1	
TX00-0035	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	<LOD		1	
TX00-0036	Matagorda Bay System, Matagorda Bay	Atlantic Croaker	0.3	0.3	1	
TX00-0037	San Antonio Bay System, Shoalwater Bay	Catfish	<LOD		1	
TX00-0038	San Antonio Bay System, San Antonio Bay	Penaeid Shrimp	0.5	0.4	1	
TX00-0039	Aransas Bay System, Mesquite Bay	Gafftop Sail Catfish/Atlantic Croaker	0.1	0.1	2	<LOD - 0.2
TX00-0040	Corpus Christi Bay System, Corpus Christi Bay	Silver Perch	<LOD		1	
TX00-0041	Corpus Christi Bay System, Corpus Christi Bay	Atlantic Croaker/Pinfish	0.3	0.1	2	<LOD - 0.4
TX00-0042	Upper Laguna Madre System, Upper Laguna Madre	Silver Perch	<LOD		1	
TX00-0043	Upper Laguna Madre System, Baffin Bay	Atlantic Croaker	0.5	0.3	1	
TX00-0044	Upper Laguna Madre System, Upper Laguna Madre	Pinfish	<LOD		1	
TX00-0045	Upper Laguna Madre System, Upper Laguna Madre	Pinfish	<LOD		1	
TX00-0046	Upper Laguna Madre System, Baffin Bay	Catfish	<LOD		1	
TX00-0047	Lower Laguna Madre System, Lower Laguna Madre	Catfish	<LOD		1	
TX00-0048	Lower Laguna Madre System, Lower Laguna Madre	Pigfish	<LOD		1	
TX00-0049	Lower Laguna Madre System, Lower Laguna Madre	Toadfish	<LOD		1	
TX00-0050	Lower Laguna Madre System, Lower Laguna Madre	Pinfish	<LOD		1	

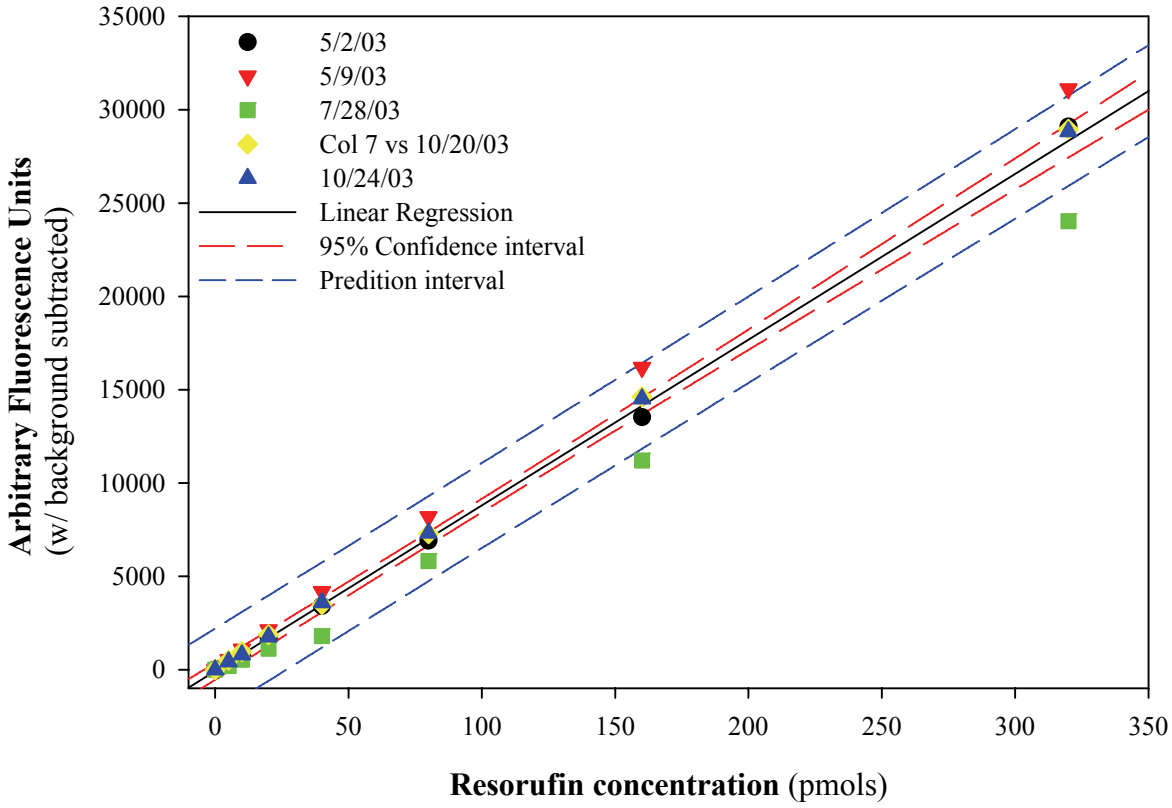

Figure 1. Resorufin standard chart. All resorufin standards run with Texas EMAP samples.

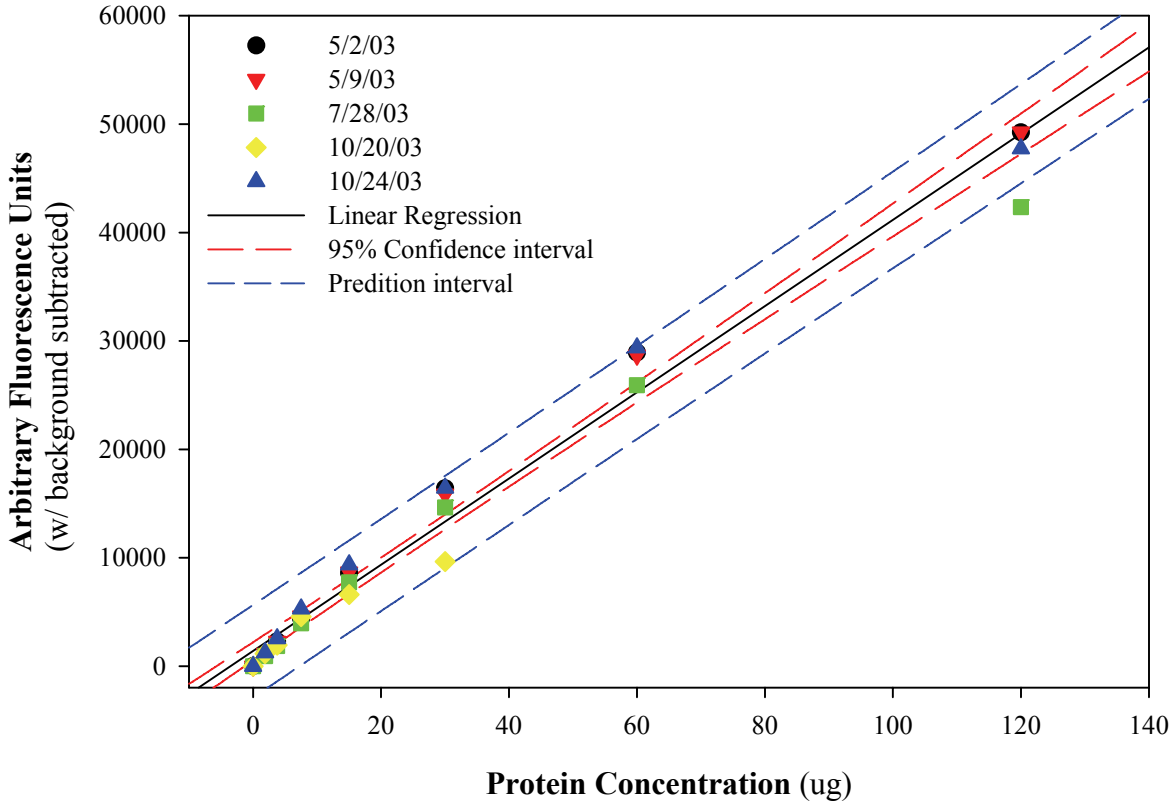

Figure 2. BSA (protein) standard chart. All bovine serum albumin (BSA) standard curves run with TX EMAP samples.

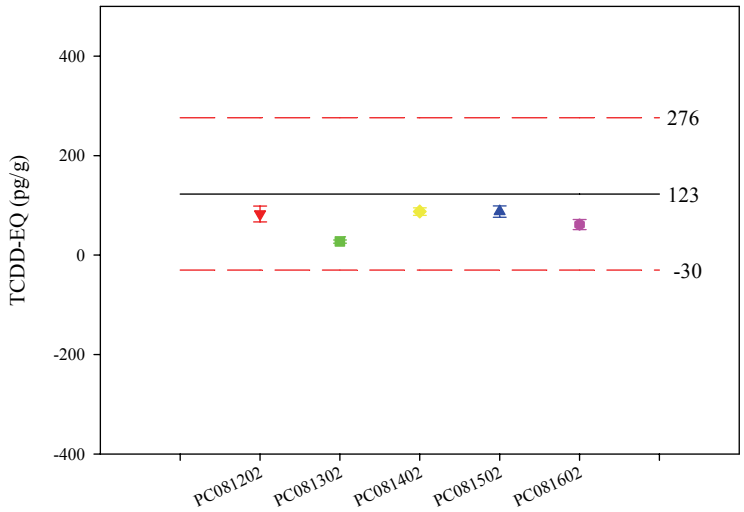

Figure 3. Positive control, control chart. Mean positive control (PC) 2,3,7,8-TCDD equivalents (TCDD-EQ) ± SD of Saginaw Bay carp PC samples assayed with the Texas EMAP samples. Sample mean values are plotted with a solid line indicating the lab mean PC TCDD-EQ value and dashed lines indicating the upper and lower confidence limits. Confidence limits were calculated using the lab mean TCDD-EQ ± two times the standard deviation.

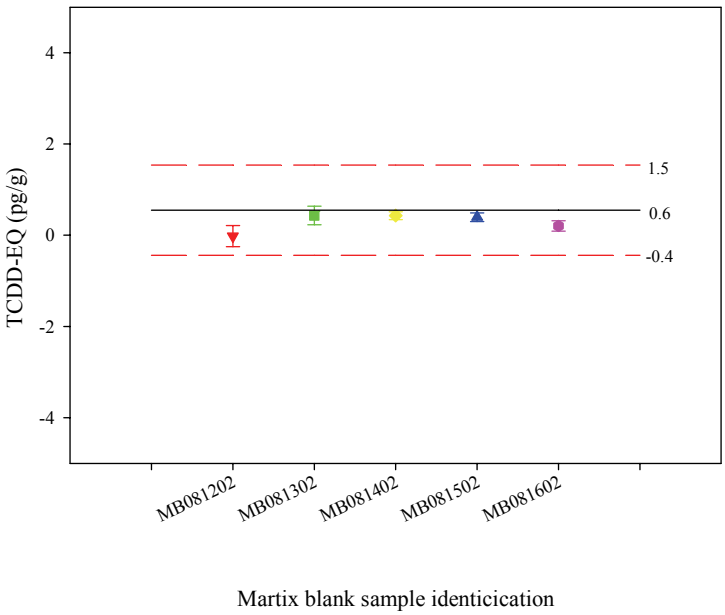

Figure 4. Matrix blank control chart. Mean matrix blank (MB) 2,3,7,8-TCDD equivalents (TCDD-EQ) ± SD of CERC bluegill matrix blank samples assayed with the Texas EMAP samples. Sample mean values are plotted with a solid line indicating the lab mean MB TCDD-EQ value and dashed lines indicating the upper and lower confidence limits. Confidence limits were calculated using the lab mean TCDD-EQ ± two times the standard deviation.

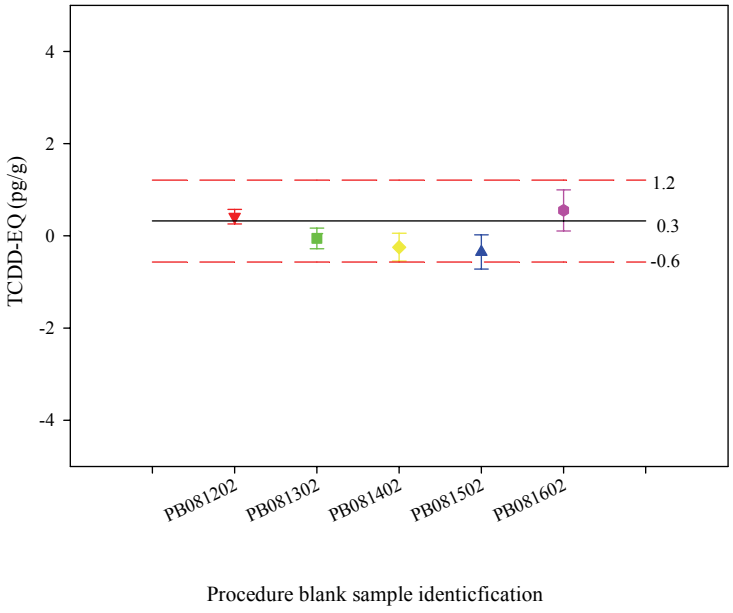

Figure 5. Procedure blank control chart. Mean procedure blank (PB) 2,3,7,8-TCDD equivalents (TCDD-EQ) ± SD of PB samples assayed with the Texas EMAP samples. Sample mean values are plotted with a solid line indicating the lab mean PB TCDD-EQ value and dashed lines indicating the upper and lower confidence limits. Confidence limits were calculated using the lab mean TCDD-EQ ± two times the standard deviation.

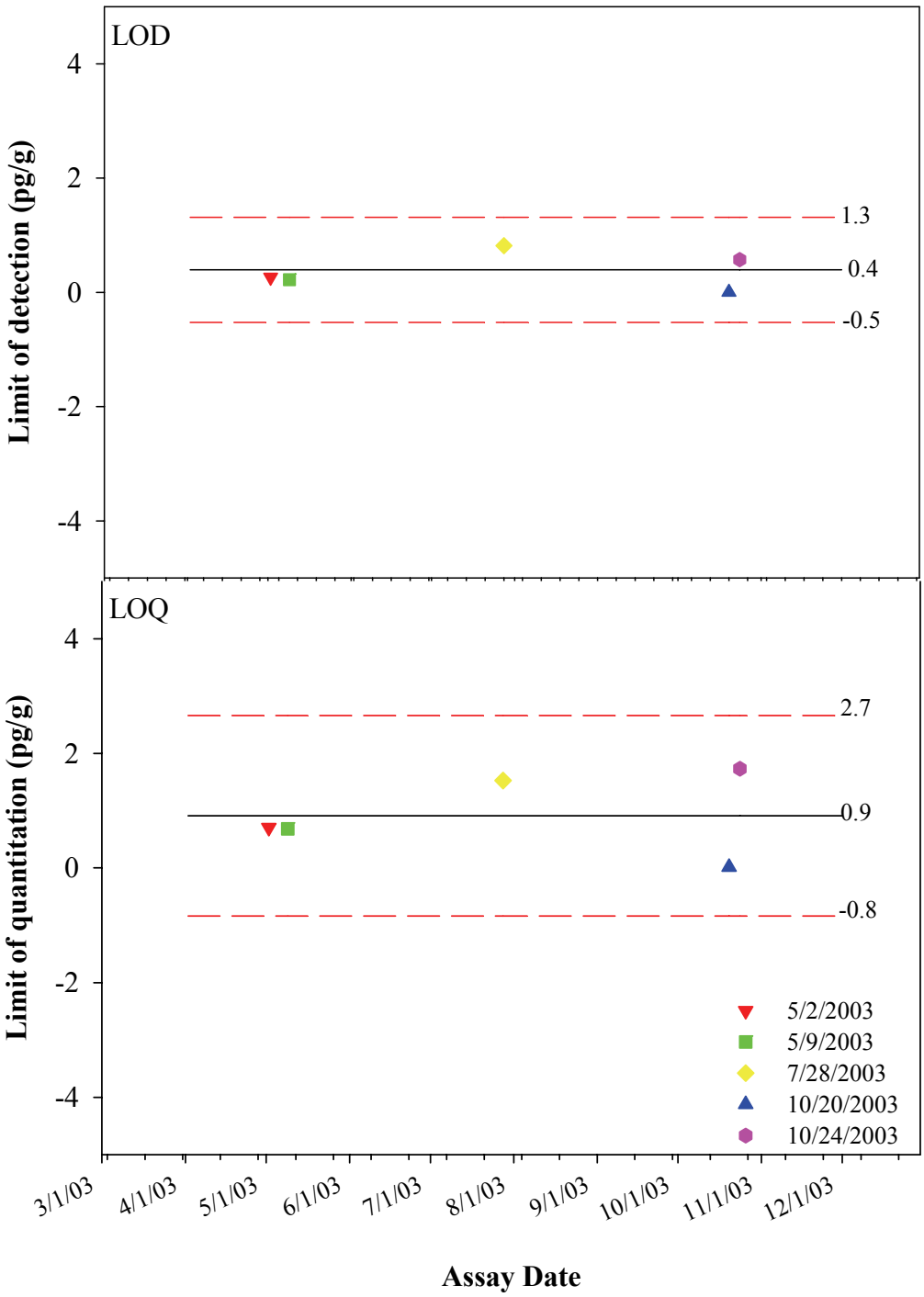

Figure 6. Limit of detection (LOD) and limit of quantitation (LOQ) graph.
Daily assay LODs or LOQs plotted with the lab mean LOD or LOQ (solid black line), upper confidence limit and lower confidence limit (dashed red lines). Confidence limits are the mean plus or minus two times the standard deviation.

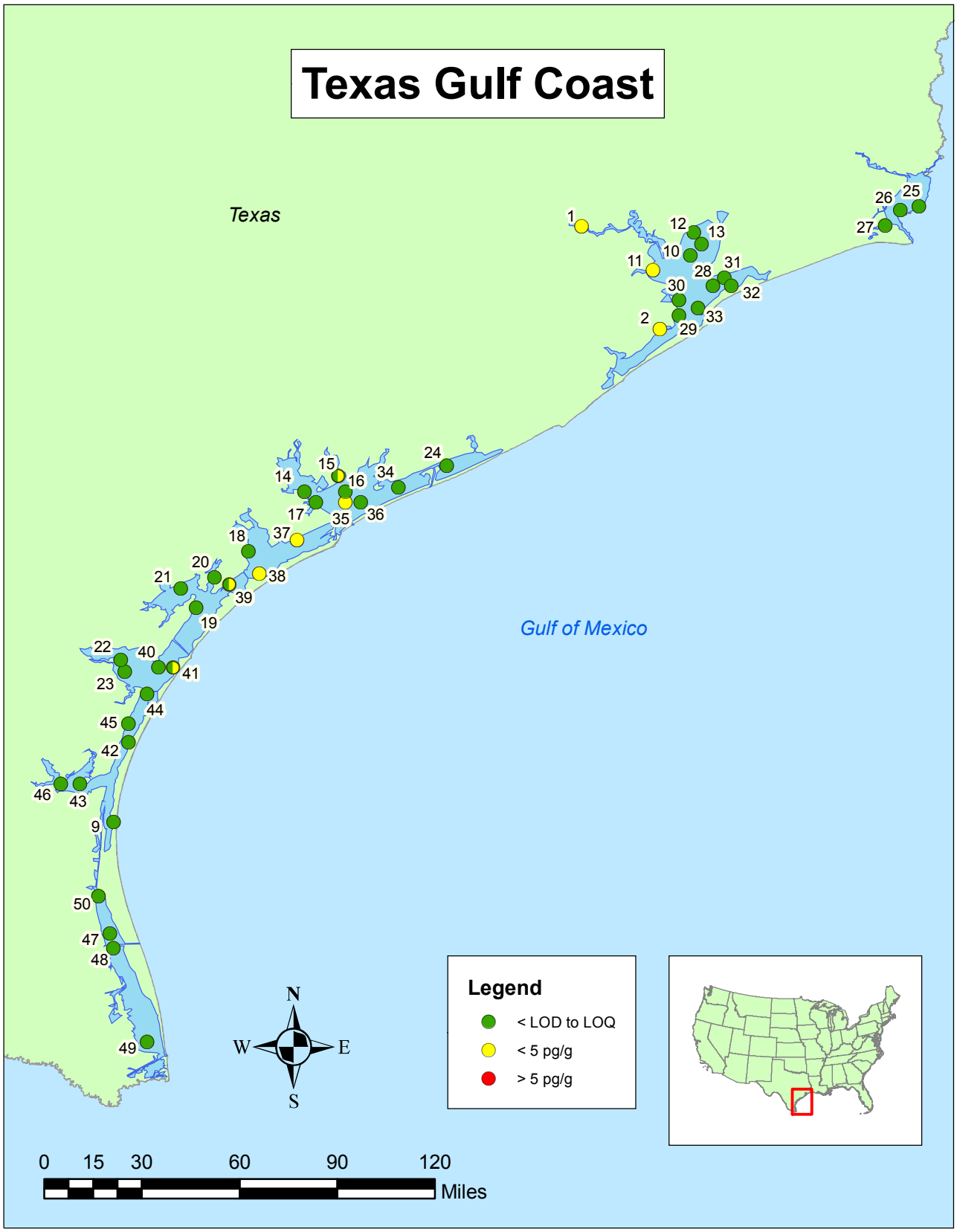

Figure 7. H4IIE bioassay-derived TCDD-EQ results for EMAP sites along the Texas Gulf Coast.